

126. Biochemical and Structural Studies of Enzymes Involved in Fatty Acid Biosynthesis

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Project Goals: Direct replacement of petroleum-derived gasoline and diesel fuels may be possible through metabolic engineering of microbial fatty acid biosynthetic pathways. We have therefore structurally and biochemically characterized two key enzymes with the goal of aiding the production of fatty acid derived biofuels.

a) *Micrococcus luteus* is a Gram-positive bacterium that produces branched alkenes, which are potential biofuels, by the condensation of fatty-acid thioesters. In an effort to better understand the control of the formation of branched fatty acids the structure of FabH was determined [1]. This enzyme catalyzes the initial step of fatty-acid biosynthesis, which is the condensation of malonyl-ACP with an acyl-CoA. Analysis of the structure provides insights into substrate selectivity with respect to length and branching of the acyl-CoA. The most structurally divergent region of FabH is a loop region located at the dimer interface, which is involved in the formation of the acyl-binding channel, limiting the substrate-channel size. A phenylalanine residue that is positioned near the catalytic triad appears to play a major role in branched-substrate selectivity. In addition to structural studies, transcriptional studies were also performed, focusing on an increase in the ratio of *anteiso*- to *iso*-branched alkenes observed at different stages of bacterial growth.

b) In general, anaerobic culture conditions would be preferred in the fermentative production of biofuels, to avoid maintaining dissolved oxygen levels and to maximize the proportion of reducing equivalents directed to biofuel. One concern with such fermentative growth conditions is increased NADH levels, which can adversely affect cell physiology. Bioinformatic and crystallographic analyses were used to identify four potential NADH-dependent variants of *Escherichia coli* FabG, an essential reductase involved in fatty acid biosynthesis [2]. Assays of cofactor preference in the variants showed up to a 35-fold preference for NADH (over NADPH), which was observed with the enzyme from *Cupriavidus taiwanensis*. In addition, use of these NADH-dependent variants in fatty acid- and methyl ketone-overproducing *E. coli* host strains under anaerobic conditions led to increased free fatty acid and methyl ketone titer relative to the controls. With further engineering, these variants could serve as a starting point for establishing a microbial host strain for production of fatty acid-derived biofuels under anaerobic conditions.

References

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- [2] Javidpour P, Pereira JH, Goh EB, McAndrew RP, Ma SM, Friedland GD, Keasling JD, Chhabra SR, Adams PD, Beller HR: Biochemical and Structural Studies of NADH- Dependent FabG Used to Increase the Bacterial Production of Fatty Acids under Anaerobic Conditions. *Appl Environ Microbiol.* 2014, **80**:497-505.

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